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Morpho-physiological attributes of wheat (*Triticum aestivum* L.) genotypes as influenced by osmo-protectants and antioxidants under terminal heat stress

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Abstract
Heat and drought cause yield reduction in wheat when there is rise in temperature during reproductive phase. The experiment was conducted at the field experimental area of Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The treatment of (Salicylic acid @ 50 µg ml^{-1}& 75 µg ml^{-1}, KNO_3 @ 0.5 & 1.0 % and $ZnSO_4.7H_2O$ @ 0.5 & 1.0%) and treatment of (Ascorbic acid @ 100 µg ml^{-1} & 200 µg ml^{-1} and Arginine @ 1.0 mM & 2.0 mM) was given excluding control and water treatment to three varieties namely PBW 550, PBW 621 and WH 1105 in a randomized block design.Heat stress adversely affects the morphophysiological traits viz., emergence, canopy temperature, plant height and stomatal distribution. Foliar spray of osmo-protectants and antioxidants was done at anthesis and 10 days after anthesis stage to ameliorate terminal heat stress in wheat.

Keywords: Osmo-protectants, antioxidants, wheat, foliar spray, salicylic acid

Introduction
Wheat (*Triticum aestivum* L.) is the most widely cultivated cereal in the world and it has a prominent position in the international food grain trade. It is the second most important grain crop in India next to rice and was grown on an area of 31.19 million hectares with a production of 95.85 million tonnes. In Punjab, it was grown on 35.38 lac hectares with a production of 16.80 million tonnes.

High temperature stress in wheat during reproductive development is a primary constraint to its production. Formation of ROS is related to ethylene production and lipid peroxidation and results in membrane fluidity (Weckx *et al* 1989) [13]. Increased ethylene has been shown in mature wheat plants, to shorten the grain filling period, decrease 1000 kernel weight, hasten maturity and trigger premature senescence (Beltrano *et al* 1999) [1]. Ethylene overproduction has also been found during or after recovery from water stress (Beltrano *et al* 1999) [1]. Don *et al* (2005) [2] reported that high temperature effects the high molecular weight fraction of gluten protein in wheat. They reported significant effects of prolonged exposure to high temperatures (up to 40ºC) on gluten in macropolymer (GMP) and its constituting gluten in particles and concluded that changes in dough mixing requirements were directly related to changes in gluten in macropolymer.

Wheat crop is found to be sensitive to temperature during reproductive phase because it may affect the source – sink relationship and as a result the yield decreases. According to a report of the Intergovernmental Panel on Climatic Change (IPCC 2007) [6] the global mean temperature will rise 0.2 ºC per decade in the coming years. This change in global temperature may alter the geographical distribution and growing season of agricultural crops (Porter 2005). High temperatures stress (≤ 40ºC) can cause scorching of leaves and twigs, sunburns on leaves, branches and stems, leaf senescence and abscission, shoot and root growth inhibition, fruit discoloration and damage and reduced yield in plants (Vollenweider and Gunthardt-Goerg 2005) [11]. International Heat Stress Genotype Experiment (IHSGE) involves the evaluation of potential physiological screening techniques by observing genetic diversity for trait and their association with heat tolerance. Canopy temperature depression (CTD) and flag leaf stomatal conductance, as well as photosynthetic rate, were highly correlated with field performance at a number of international locations (Reynolds *et al* 2000) [8]. The apparent sensitivity of metabolic processes to heat stress in the field (Reynolds *et al* 2000) [8], coupled with the reduced length of life cycle at high temperature determines total plant biomass and ultimately grain yield in hot environments.

Wheat yield in such conditions could be improved,by modifying the crop micro-climate through some cultural practices and their optimum use such as time of sowing, mulching, frequent irrigation, ethylene inhibitors, osmoprotectants and nutrients (Dupont *et al* 2006) [3].
Among the various methods to induce high temperature stress in plant, foliar application of, or pre-sowing seed treatment with, low concentrations of inorganic salts, osmo-protectants, signalling molecules (e.g., growth hormones) and oxidants (e.g H_2O_2) as well as preconditioning of plants are common approaches (Wahid *et al* 2007) [12]. Osmo-protectants also stimulate photosynthetic machinery and cell division and hence improve the yield and quality of crops.Foliar application of salicylic acid at 50mg L^{-1} and KNO_3 at 2% recorded the maximum heads m^{-2}, seeds $head^{-1}$, 1000-seed weight, seed yield and seed quality in berseem which were significantly higher than the control (Kumar *et al* 2013) [7].
Chemicals like cobalt chloride, potassium nitrate and calcium chloride have a role in terminal heat stress management in wheat (Sarlach *et al* 2013) [9]. Ascorbic acid and α-tocopherol sprayed plants postponed the leaf senescence by peroxide/ phenolic/ ascorbate system which is involved in scavenging the reactive oxygen species (ROS) produced during leaf senescence.Accumulation of ROS as a result of high temperature stress is a major cause of loss of crop productivity worldwide. (Tuteja 2010) [10]. The present investigation was undertaken with the objective to characterize morpho physiological traits and to evaluate the performance of different osmo-protectants and antioxidants to elevated temperatures during the reproductive and grain-filling stages:

Methodology

The present investigation was carried out in experimental area, Wheat section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The field experiment was conducted at experimental area of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana in loamy soil. The bulk density of soil was 1.6 gm/cubic cm, low in potassium, optimum in phosphorus and moderate in nitrogen. The pH of soil was 7.8.The crop was sown in randomized block design with three replications using 100 kg seed rate per hectare at 20.0 cm row spacing. One third nitrogen, full phosphorus and potash were applied below the seed at the time of sowing and were sown by *kera* (dropping of seed in the open furrow). The seed was treated with thiram @ 1.0g/kg seed against the seed borne diseases. The weeds were controlled by spraying weedicide and the crop was irrigated five times. Two foliar sprays of osmo-protectants (Salicylic acid, KNO_3 and $ZnSO_4.7H_2O$) and antioxidants (Ascorbic acid and arginine) were done when the crop reached at anthesis stage and ten days after anthesis stage. Data on emergence count, periodic plant height, number of tillers per meter row length, days to anthesis, days to maturity, canopy temperature and stomatal frequencywere recorded. The Chlorophyll content was recorded with chlorophyll content meter (Model CCM 200) starting from anthesis to maturity at regular interval of time while canopy temperature was recorded first at anthesis stage and then after 10 days by using infrared thermometer (Model LT-300).

Varieties : 3 (PBW 550, PBW 621 and WH 1105)
Replications : 3
Chemicals : 3- Salicylic acid, KNO_3 and $ZnSO_4.7H_2O$
2- Ascorbic acid and Arginine

Statistical analysis

The statistical analysis of the data of morpho-physiological and yield related parameters was done using a factorial randomized block design. Critical differences (CD) were computed at 5% probability level. The statistical analysis was carried out with the help of CPCS-1 software and the results so obtained are discussed accordingly.

Results and discussion

Morpho-physiological attributes of wheat as affected under heat stress

Emergence count

It was observed that there was not much difference in emergence count within varieties and treatments. The data (Table 1) showed non-significant interaction between varieties. Treatments of osmo-protectants were given at the time of anthesis so treatments had no effect on emergence count per plot. Emergence count was calculated only to check the uniformity among the plots of varieties.
It was concluded from the data (Table 2) that there was not much difference in emergence count within varieties and treatments. The data showed non-significant interaction due to not as much difference between varieties. Treatment of antioxidants were given at the time of anthesis so treatments had no effect on emergence count per plot.

Days to anthesis

Days to anthesis is a pivotal trait in wheat cultivars and it is reported that increase in days to flowering reduces the grain filling period under heat stress and grain filling period has a strong influence on grain yield *via* grain weight (Simane *et al* 1993).
The maximum days to anthesis (DTA) was taken by PBW 621 (97.9 days) and minimum was taken by PBW 550 (89.4 days) and average days to anthesis was taken by WH 1105 (92.9 days). Treatment was given at the time of anthesis, therefore, had no effect on days to anthesis (Table 1). Days to anthesis is a pivotal trait in wheat cultivars and it reported that increase in days to flowering reduces the grain filling period under heat stress and grain filling period has a strong influence on grain yield *via* grain weight (Simane *et al* 1993).
Under non-treatment condition, maximum days to anthesis (DTA) was taken by PBW 621 (97.8 days) and mimimum was taken by PBW 550 (90.1 days) and average days to anthesis was recorded in WH 1105 (94.2 days). Treatment was given at the time of anthesis therefore, had no effect on days to anthesis (Table 2).

Days to maturity

Under treatment of osmo-protectants, maximum days to maturity (DTM) was reported in PBW 621 (142.3 days), followed by WH 1105 (137.8 days) and PBW 550 (133.9 days). Both the concentrations of KNO_3 showed more days to maturity period than other treatments. Other foliar sprays were affected as similar as control (Table 1). The maximum days to maturity (DTM) was taken by PBW 621 (142.3 days), followed by WH 1105 (141.1 days) and mimimum was taken by PBW 550 (133.9 days). Treatment was given at the time of anthesis therefore, had effect on days to maturity. Both the concentrations of ascorbic acid showed more days to maturity period than other treatments. Other foliar sprays were as par with control (Table 2).

Table 1: Effect of different osmo-protectants on emergence count per plot, days to anthesis and days to maturity in wheat (*Triticum aestivum* L.)

Treatments	Emergence count per plot				Days to anthesis				Days to maturity			
	PBW 550	**PBW 621**	**WH 1105**	**Mean**	**PBW 550**	**PBW 621**	**WH 1105**	**Mean**	**PBW 550**	**PBW 621**	**WH 1105**	**Mean**
T1:- Salicylic acid (50µg ml^{-1})	410.0	458.7	446.7	438.4	83.3	98.0	92.0	91.1	133.7	141.0	137.7	137.4
T2:- Salicylic acid (75µg ml^{-1})	432.7	428.0	446.0	435.6	91.7	97.3	94.0	94.3	134.0	143.3	132.0	136.4
T3:- KNO_3 (0.5%)	485.3	404.0	406.0	431.8	89.3	99.0	90.7	93.0	142.7	146.0	145.7	144.8
T4:- KNO_3 (1%)	449.3	436.0	430.7	438.7	90.0	96.3	91.7	92.7	141.0	143.7	146.3	143.7
T5:- $ZnSO_4.7H_2O$ (0.5%)	414.0	422.0	439.3	425.1	87.0	101.0	94.0	94.0	132.7	140.0	132.7	135.1
T6:- $ZnSO_4.7H_2O$ (1%)	421.3	438.7	438.7	432.9	87.7	95.0	92.7	91.8	128.7	140.0	133.3	134.0
T7:- Water sprayed	461.3	430.0	436.7	442.7	94.0	97.3	93.3	94.9	128.3	144.7	142.7	138.6
T8:- Unsprayed (control)	407.3	422.7	415.3	415.1	92.3	99.3	95.0	95.6	130.0	140.0	132.3	134.1
Mean	435.2	430.0	432.4	432.5	89.4	97.9	92.9	93.4	133.9	142.3	137.8	138.0
CD 5%	Varieties (A) = NS Treatments (B) = NS A x B = NS				Varieties (A) = 4.8 Treatments (B)= NS A x B = NS				Varieties (A) = 5.5 Treatments (B) = 6.7 A x B = NS			

Table 2: Effect of different antioxidants on emergence count per plot, days to anthesis and days to maturity in wheat (*Triticum aestivum* L.)

Treatments	Emergence count per plot				Days to Anthesis				Days to Maturity			
	PBW 550	**PBW 621**	**WH 1105**	**Mean**	**PBW 550**	**PBW 621**	**WH 1105**	**Mean**	**PBW 550**	**PBW 621**	**WH 1105**	**Mean**
T1- (Ascorbic acid 100 µgml^{-1})	385.3	458.7	422.7	422.2	89.3	98.0	99.3	95.6	142.7	141	144.7	142.8
T2- (Ascorbic acid 200 µgml^{-1})	449.3	428.0	430.0	435.8	90.0	97.3	97.3	94.9	141.0	143.3	140.0	141.4
T3- (Arginine 1.0 mM)	414.0	404.0	446.7	421.6	87.0	99.0	92.0	92.7	132.7	146.0	137.7	138.8
T4- (Arginine 2.0 mM)	421.3	436.0	446.0	434.4	87.7	96.3	94.0	92.7	128.7	143.7	132.0	134.8
T5- (Water sprayed)	407.3	422.0	406.0	411.8	92.3	101.0	90.7	94.7	128.3	140.0	145.7	138.0
T6- (Unsprayed control)	461.3	438.7	430.7	443.6	94.0	95.0	91.7	93.6	130.0	140.0	146.3	138.8
Mean	423.1	431.2	430.3	428.2	90.1	97.8	94.2	94.0	133.9	142.3	141.1	139.1
CD at 5%	Varieties (A) = NS Treatments (B) = NS A x B =NS				Varieties (A) = 5.1 Treatments (B) = NS A x B = NS				Varieties (A) = 4.6 Treatments (B) = NS A x B = NS			

Periodic Plant height

Plant height is influenced by the interaction of environmental conditions and genetic constitution of the plant. Though plant height is genetically controlled, but water stress also plays an important role in its regulation. Plant height is among one of the most important characters in wheat production.

Maximum plant height was recorded on 60 DAS, 90 DAS, 120 DAS and at harvest in PBW 621 (46.2 cm, 73.4 cm, 94.9 cm and 97.4 cm respectively) and minimum plant height in PBW 550 (40.0cm, 64.9 cm, 74.2 cm and 74.4 cm respectively) at harvest. The non-significant difference was observed between varieties and foliar sprays of different osmo-protectants at 60, 90 DAS, 120 DAS and at harvest (Table 3). Plant height is influenced by the interaction of environmental conditions and genetic constitution of the plant. Though plant height is genetically controlled, but heat stress also plays an important role in its regulation. Plant height is among one of the most important characters in wheat production.

Maximum plant height was recorded on 60 DAS, 90 DAS, 120 DAS and at harvest in PBW 621 (46.2 cm, 73.4 cm, 94.9 cm and 97.4 cm respectively) and minimum plant height in PBW 550 (40.0 cm, 64.9 cm, 74.2 cm and 74.4 cm respectively) at harvest. The non-significant difference was observed among the treatments at 60 and 90 DAS and significant difference was recorded on 120 DAS and at harvest. Ascorbic acid (100 µg ml^{-1}) showed maximum mean of plant height and minimum mean (82.3 cm) was recorded on the influence of water sprayed at 120 DAS and (88.3 cm) under water spray and control environment. There was a non-significant interaction recorded on periodic plant height among varieties and foliar sprays (Table 4).

Canopy temperature (CT)

Canopy temperature recognized as indicators of overall plant water statusand used in such practical applications as evaluation of plant response to environmental stress like tolerance to heat (Reynolds *et al* 2000) [8]. For performance of different osmo-protectants, minimum canopy temperature was recorded in PBW 550 (18.1 °C) and maximum was found in WH 1105 (25.9 °C), followed by PBW 621 (25.9 °C) at anthesis. 10 days after anthesis, average CT was found in PBW 621 and PBW 550 (21.1 °C) and maximum was recorded in WH 1105 (28.0 °C) (Table 5). Non-significant difference was recorded among foliar sprays on CT at both (at anthesis and 10 days after anthesis). Non-significant Interaction between varieties and foliar sprays on CT at anthesis and 10 days after anthesis were recorded.

For performance of different antioxidants, minimum canopy temperature was recorded in PBW 550 (18.1 °C) and maximum was found in WH 1105 (24.1 °C) at anthesis. 10 days after anthesis minimum CT was found in PBW 550 (21.2 °C) and maximum was recorded in WH 1105 (28.1 °C), while PBW 621 showed average CT at anthesis (20.5 °C) and (23.5 °C) 10 days after anthesis.

It was observed that under foliar spray condition, both the concentrations of ascorbic acid (100 µgml^{-1}), followed by (200 µg ml^{-1}) recorded minimum CT at anthesis and 10 days after anthesis (19.2 ^{0}C, 19.9 ^{0}C and 23.1 ^{0}C, 23.3 ^{0}C) respectively (Table 6).

Table 3: Effect osmo-protectants on periodic plant height in wheat (*Triticum aestivum* L.)

Treatments	Periodic Plant Height (cm)															
	60 DAS				90 DAS				120 DAS				At harvest			
	PBW 550	PBW 621	WH 1105	Mean	PBW 550	PBW 621	WH 1105	Mean	PBW 550	PBW 621	WH 1105	Mean	PBW 550	PBW 621	WH 1105	Mean
T1:- Salicylic acid (50µg ml^{-1})	39.0	47.0	43.0	43.0	65.0	77.0	79.0	73.7	72.7	86.0	94.0	84.2	80.0	98.0	95.0	91.0
T2:- Salicylic acid (75µg ml^{-1})	38.0	47.0	46.0	43.7	75.0	76.0	69.0	73.3	74.0	87.0	89.0	83.3	77.0	96.0	94.0	89.0
T3:- KNO_3 (0.5%)	38.0	47.0	46.0	43.7	68.0	70.0	74.0	70.7	72.0	91.0	82.0	81.7	78.0	98.0	90.0	88.7
T4:- KNO_3 (1%)	38.0	47.0	47.0	44.0	64.0	71.0	72.0	69.0	73.0	94.0	88.0	85.0	75.0	96.0	94.0	88.3
T5:- $ZnSO_{4.7}$H2O (0.5%)	42.0	47.0	48.0	45.7	68.0	71.0	71.0	70.0	71.0	93.0	90.0	84.7	76.0	97.0	90.0	87.7
T6:- $ZnSO_4.7H_2O$ (1%)	45.0	46.0	45.0	45.3	66.0	75.0	75.0	72.0	70.0	97.0	89.0	85.3	76.0	98.0	93.0	89.0
T7:- Water sprayed	38.0	49.0	48.0	45.0	65.0	79.0	70.0	71.3	74.0	93.0	87.0	84.7	74.0	95.0	90.0	86.3
T8:- Unsprayed (control)	43.0	45.0	48.0	45.3	67.0	74.0	72.0	71.0	73.0	98.0	84.0	85.0	76.0	99.0	88.0	87.7
Mean	40.1	46.9	46.5	44.5	67.3	74.1	72.8	71.4	72.4	92.4	87.9	84.2	76.5	97.13	91.8	88.5
CD at 5%	Varieties (A) = 1.9 Treatments (B) = NS A x B = NS				Varieties (A) = 3.1 Treatments (B) = NS A x B = NS				Varieties (A) = 3.5 Treatments (B) = NS A x B = NS				Varieties (A) = 3.6 Treatments (B) = NS A x B = NS			

Table 4: Effect of ascorbic acid and arginine on periodic plant height in wheat (*Triticum aestivum* L.)

Treatments	Periodic Plant Height (cm)															
	60 DAS				90 DAS				120 DAS				At Harvest			
	PBW 550	PBW 621	WH 1105	Mean	PBW 550	PBW 621	WH 1105	Mean	PBW 550	PBW 621	WH 1105	Mean	PBW 550	PBW 621	WH 1105	Mean
T1- (Ascorbic acid 100 µgml^{-1})	43.0	46.7	45.0	44.9	68.0	77.3	74.0	73.1	76.3	95.7	98.0	90.4	77.7	98.0	97.3	90.6
T2- (Ascorbic acid 200 µgml^{-1})	38.3	47.0	49.3	44.9	64.0	76.0	79.0	73.0	73.3	93.7	89.7	86.8	75.3	96.3	93.3	87.1
T3- (Arginine 1.0 mM)	42.0	46.7	43.3	44.0	68.3	69.7	78.7	72.2	75.7	98.7	93.7	89.3	75.7	98.3	93.7	89.2
T4- (Arginine 2.0 mM)	45.3	47.0	45.7	46.0	66.3	70.7	69.3	68.8	73.0	97.7	89.0	87.4	75.6	99.3	94.3	88.9
T5- (Water sprayed)	38.0	44.0	39.3	40.4	61.7	71.3	70.3	67.8	72.0	92.7	82.3	82.3	72.7	97.3	95.0	88.3
T6- (Unsprayed control)	38.3	46.0	38.3	40.9	61.3	75.3	75.0	70.6	70.7	91.3	88.3	83.4	73.7	95.0	96.3	88.3
Mean	40.8	46.2	43.5	43.5	64.9	73.4	74.4	70.9	74.2	94.9	90.8	86.6	74.4	97.4	94.4	88.7
CD at 5%	Varieties (A) = 2.4 Treatments (B) = NS A x B = NS				Varieties (A) = 2.8 Treatments (B) = NS A x B = NS				Varieties (A) = 2.8 Treatments (B) = 4.0 A x B = NS				Varieties (A) = 3.7 Treatments (B) = 4.1 A x B = NS			

Table 5: Effect of different concentrations of osmo-protectants on canopy temperature at anthesis and 10 days after anthesis in wheat (*Triticum aestivum* L.)

Treatments	Canopy Temperature (^{0}C)							
	At anthesis				10 days after anthesis			
	PBW 550	PBW 621	WH 1105	Mean	PBW 550	PBW 621	WH 1105	Mean
T1:- Salicylic acid (50µg ml^{-1})	18.0	20.0	25.0	21.0	21.0	28.0	21.0	23.3
T2:- Salicylic acid (75µg ml^{-1})	18.0	21.0	26.0	21.7	21.0	28.0	21.0	23.3
T3:- KNO_3 (0.5%)	18.0	21.0	26.0	21.7	21.0	27.0	21.0	23.0
T4:- KNO_3 (1%)	18.0	21.0	26.0	21.7	21.0	28.0	21.0	23.3
T5:- $ZnSO_4.7H_2O$ (0.5%)	18.0	20.0	26.0	21.3	22.0	28.0	21.0	23.7
T6:- $ZnSO_4.7H_2O$ (1%)	18.0	20.0	26.0	21.3	21.0	29.0	22.0	24.0
T7:- Water sprayed	19.0	21.0	26.0	22.0	21.0	28.0	21.0	23.3
T8:- Unsprayed (control)	18.1	20.5	25.9	21.5	21.1	28.0	21.1	23.4
Mean	18.0	20.0	26.0	21.3	21.0	28.0	21.0	23.3
CD at 5%	Varieties (A) = 0.4 Treatments (B) = NS A x B = NS				Varieties (A) = 0.3 Treatments (B) = NS A x B = NS			

Table 6: Effect of different concentrations of antioxidants on canopy temperature at anthesis and 10 days after anthesis in wheat (*Triticum aestivum* L.)

Treatments	Canopy Temperature (^{0}C)							
	At anthesis				10 day after anthesis			
	PBW 550	PBW 621	WH 1105	Mean	PBW 550	PBW 621	WH 1105	Mean
T1- (Ascorbic acid 100 µgml^{-1})	17.9	20.6	21.1	19.9	20.6	21.3	27.9	23.3
T2- (Ascorbic acid 200 µgml^{-1})	17.7	19.6	20.1	19.2	20.5	21.0	27.7	23.1
T3- (Arginine 1.0 mM)	18.1	20.5	25.5	21.4	21.3	21.3	27.9	23.5
T4- (Arginine 2.0 mM)	18.2	20.6	25.6	21.5	21.4	21.4	28.2	23.6
T5- (Water sprayed)	18.2	20.7	26.0	21.7	21.6	28.0	28.2	25.9
T6- (Unsprayed control)	18.5	20.7	26.3	21.8	21.8	28.1	28.4	26.1
Mean	18.1	20.5	24.1	20.9	21.2	23.5	28.1	24.3
CD at 5%	Varieties (A) = 0.6 Treatments (B) = 0.8 A x B = 1.4				Varieties (A) = 0.4 Treatments (B) = 0.5 A x B = 0.9			

Chlorophyll content

Chlorophyll is one of the major chloroplast components for photosynthesis, and relative chlorophyll content has a positive relationship with photosynthetic rate. The decrease in chlorophyll content under heat stress has been considered a typical symptom of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation (Farooq *et al* 2011) [4].

The maximum chlorophyll content was recorded in PBW 550 (31.6) and minimum was found in PBW 621 (24.0) at anthesis stage. After 5 days of anthesis maximum chlorophyll content was again recorded in PBW 550 (32.1) and minimum was recorded in PBW 621 (25.0). After 10 days of anthesis maximum chlorophyll content was again found in PBW 550 (29.8) and minimum was found in PBW 621 (24.3). PBW 621 showed maximum chlorophyll content at 15, 20, 25 and 30 days after anthesis (18.5, 15.1, 10.75, 3.7 respectively) and minimum chlorophyll content was recorded in PBW 550 (14.3, 12.6, 6, 1.1 respectively). Average chlorophyll content was recorded in WH 1105 starting from anthesis to 30 days after anthesis (28.9, 27.0, 24.9, 14.4, 14.1, 8.5 and 2.1) (Table 7a and 7b). Significant interactions were recorded on 10, 20 and 25 days after anthesis and non-significant interactions were recorded in rest of the days of data collected after anthesis. Salicylic acid might be involved in mobilization of internal tissue NO^{3-} and chlorophyll biosynthesis to increase the functional state of the photosynthetic machinery in plants (Hayat *et al* 2010).

For foliar application of antioxidants, maximum chlorophyll content was recorded in PBW 550 (31.6) and minimum was found in PBW 621 (24.3) at anthesis stage. After 5 days of anthesis maximum chlorophyll content was again recorded in PBW 550 (31.7) and minimum was recorded in PBW 621 (25.1). After 10 days of anthesis maximum chlorophyll content was again found in PBW 550 (29.3) and minimum was found in WH 1105 (24.7). PBW 621 showed maximum chlorophyll content at 15, 20, 25 and 30 days after anthesis (18.9, 16.3, 10.7, 3.7 respectively) and minimum chlorophyll content was recorded in PBW 550 (14.8, 12.2, 5.75, 1.0 respectively). Significant interactions were recorded between varieties and treatments on chlorophyll content starting from anthesis to maturity (Table 8a and 8b).

Effective tillers per meter row length

There were no effective tillers per meter row length were recorded on 60 DAS. Effective tillers per meter row length in PBW 550 (66.3) were recorded on 90 DAS. Maximum number of effective tillers were found in PBW 621 (100.63) and PBW 550 recorded minimum number of tillers per meter row length (95.0) and average number of effective tillers per meter row length (99.6) were recorded in WH 1105 on 120 DAS. At harvest, Maximum number of effective tillers per meter row length were found in PBW 621 (104.0), PBW 550 recorded minimum number of effective tillers per meter row length (98.6) and average number of effective tillers per meter row length (101.0) were recorded in WH 1105 (Table 9).

On 120 DAS, KNO_3 (0.5%) showed maximum effective tillers per meter row length (107.0) followed by salicylic acid at 50 µgml^{-1} (102.0) and minimum (92.0) in control. At harvest, maximum tillers (108.0) were recorder on the application of KNO_3 (0.5%), followed by Salicylic acid at 50 µgml^{-1} and minimum (93.7) by the foliar application of $ZnSO_4.7H_2O$ (1%). Non-significant interaction on effective tillers per meter row length was recorded between varieties and treatments.

There were no effective tillers per meter row length recorded at 60 DAS. Effective tillers per meter row length in PBW 550 (63.0) were recorded on 90 DAS**.** Maximum number of tillers was found in PBW 621 (99.8) and PBW 550 recorded minimum number of tillers per meter row length (95.0) and average number of tillers per meter row length (99.4) was recorded in WH 1105 on 120 DAS. At harvest, Maximum number of tillers was found in PBW 621 (104.0), PBW 550 recorded minimum number of tillers per meter row length (97.7) and average number of tillers per meter row length (101.0) was recorded in WH 1105.

On 120 DAS, arginine (1.0 mM) showed maximum effective tillers per meter row length (103.0) followed by ascorbic acid at 100 µg ml^{-1} (102.0) and minimum (91.6) on water spray. At harvest, maximum tillers (108.0) were recorder by the application of both the concentrations of arginine (1.0 mM) and ascorbic acid at 100 µg ml^{-1} and minimum (92.8) under control environment. There was non-significant interaction present between varieties and treatments (Table 10).

Table 7a: Effect of different osmo-protectants on chlorophyll content (SPAD) in wheat (*Triticum aestivum* L.)

Treatments	Chlorophyll content															
	at anthesis				5th day after anthesis				10th day after anthesis				15th day after anthesis			
	PBW 550	PBW 621	WH 1105	MEAN	PBW 550	PBW 621	WH 1105	MEAN	PBW 550	PBW 621	WH 1105	MEAN	PBW 550	PBW 621	WH 1105	Mean
T1:- Salicylic acid (50µg ml^{-1})	32.0	25.0	32.0	29.7	34.0	28.0	32.0	31.3	33.0	26.0	26.0	28.3	15.0	19.0	16.0	16.7
T2:- Salicylic acid (75µg ml^{-1})	32.0	23.0	31.0	28.7	33.0	24.0	27.0	28.0	31.0	24.0	25.0	26.7	14.0	17.0	14.0	15.0
T3:- KNO_3 (0.5%)	35.0	28.0	33.0	32.0	36.0	30.0	33.0	33.0	34.0	28.0	26.0	29.3	20.0	22.0	17.0	19.7
T4:- KNO_3 (1%)	32.0	24.0	30.0	28.7	32.0	24.0	25.0	27.0	31.0	24.0	25.0	26.7	16.0	19.0	16.0	17.0
T5:- $ZnSO_4.7H_2O$ (0.5%)	32.0	23.0	26.0	27.0	29.0	21.0	24.0	24.7	23.0	23.0	25.0	23.7	13.0	18.0	13.0	14.7
T6:- $ZnSO_4.7H_2O$ (1%)	30.0	22.0	24.0	25.3	29.0	23.0	26.0	26.0	27.0	23.0	24.0	24.7	12.0	17.0	11.0	13.3
T7:- Unsprayed (control)	29.0	23.0	27.0	26.3	31.0	24.0	24.0	26.3	28.0	22.0	24.0	24.7	13.0	18.0	13.0	14.7
T8:- Water sprayed (control)	31.0	24.0	28.0	27.7	33.0	26.0	25.0	28.0	32.0	24.0	24.0	26.7	14.0	18.0	15.0	15.7
Mean	31.6	24.0	28.9	28.2	32.1	25.0	27.0	28.0	29.9	24.3	24.9	26.3	14.6	18.5	14.4	15.8
CD at 5%	Varieties (A) = 1.4 Treatments (B) = 2.3 A x B = NS				Varieties (A) = 1.2 Treatments (B) = 1.9 A x B = NS				Varieties (A) = 1.2 Treatments (B) = 1.9 A x B = 3.4				Varieties (A) = 0.7 Treatments (B) = 1.1 A x B = NS			

Table 7b: Effect of different osmo-protectants on chlorophyll content (SPAD) in wheat (*Triticum aestivum* L.)

Treatments	Chlorophyll content											
	20th day after anthesis				25th day after anthesis				30 days after anthesis			
	PBW 550	PBW 621	WH 1105	Mean	PBW 550	PBW 621	WH 1105	MEAN	PBW 550	PBW 621	WH 1105	Mean
T1:- Salicylic acid (50µg ml^{-1})	17.0	18.0	16.0	17.0	7.0	12.0	10.0	9.7	1.3	3.7	2.3	2.4
T2:- Salicylic acid (75µg ml^{-1})	11.0	16.0	15.0	14.0	7.0	9.0	9.0	8.3	0.9	3.6	2.1	2.2
T3:- KNO_3 (0.5%)	18.0	19.0	18.0	18.3	8.0	12.0	10.0	10.0	1.5	4.1	2.6	2.7
T4:- KNO_3 (1%)	12.0	17.0	15.0	14.7	7.0	10.0	9.0	8.7	1.1	3.6	2.1	2.3
T5:- $ZnSO_4.7H_2O$ (0.5%)	9.0	11.0	12.0	10.7	5.0	11.0	8.0	8.0	0.8	3.6	1.8	2.1
T6:- $ZnSO_4.7H_2O$ (1%)	13.0	12.0	11.0	12.0	4.0	10.0	7.0	7.0	0.7	3.5	1.8	2.0
T7:- Unsprayed (control)	10.0	14.0	12.0	12.0	4.0	10.0	7.0	7.0	1.0	3.6	1.8	2.1
T8:- Water sprayed (control)	11.0	14.0	14.0	13.0	6.0	12.0	9.0	9.0	1.2	3.6	2.1	2.3
Mean	12.6	15.1	14.1	14.0	6.0	10.8	8.6	8.5	1.1	3.7	2.1	2.3
CD at 5%	Varieties (A) = 0.4 Treatments (B) = 0.6 A x B = 1.04				Varieties (A) = 0.4 Treatments (B) = 0.7 A x B = 1.2				Varieties (A) = 0.1 Treatments (B) = 0.2 A x B = NS			

Table 8a: Effect of different antioxidants on chlorophyll content (SPAD) in wheat (*Triticum aestivum* L.)

Treatments	Chlorophyll content															
	at anthesis				5th day after anthesis				10th day after anthesis				15th day after anthesis			
	PBW 550	PBW 621	WH 1105	MEAN	PBW 550	PBW 621	WH 1105	Mean	PBW 550	PBW 621	WH 1105	MEAN	PBW 550	PBW 621	WH 1105	Mean
T1- (Ascorbic acid 100 μgml^{-1})	34.8	25.2	23.3	27.8	36.4	27.7	24	29.4	34.3	26.4	21.9	27.6	19.6	19.4	18.3	19.1
T2- (Ascorbic acid 200 μgml^{-1})	32.4	23.4	24.1	26.7	32.5	24.4	26	27.6	31.4	24.6	23.9	26.6	15.8	17.5	17.9	17.1
T3- (Arginine 1.0 mM)	31.7	27.9	32.3	30.6	28.8	29.9	32.1	30.3	23.1	28.4	25.8	25.8	13.2	22.5	16.0	17.2

T4- (Arginine 2.0 mM)	30.3	23.8	30.9	28.3	28.7	24.5	26.7	26.6	26.8	24.5	25.0	25.4	12.5	18.6	14.3	15.2
T5- (Water sprayed)	29.4	23.3	33.2	28.7	30.8	20.8	32.8	28.1	28.1	23.4	26.3	25.9	13.3	18.1	16.7	16.1
T6- (Unsprayed control)	30.8	22.3	30.0	27.7	33.3	23.6	25.6	27.5	32.2	23.4	25.5	27.0	14.1	17.0	15.8	15.6
Mean	31.6	24.3	29.0	28.3	31.7	25.1	27.9	28.2	29.3	25.1	24.7	26.4	14.8	18.9	16.5	16.7
CD at 5%	Varieties (A) = 1.6 Treatments (B) = 2.2 A x B = 3.8				Varieties (A) = 1.4 Treatments (B) = 2.1 A x B = 3.5				Varieties (A) = 1.4 Treatments (B) = NS A x B = 3.5				Varieties (A) = 0.9 Treatments (B) = 1.2 A x B = 2.2			

Table 8b: Effect of different antioxidants on chlorophyll content (SPAD) in wheat (*Triticum aestivum* L.)

Treatments	Chlorophyll content											
	20th day after anthesis				25th day after anthesis				30 DAS (lower surface)			
	PBW 550	PBW 621	WH 1105	Mean	PBW 550	PBW 621	WH 1105	MEAN	PBW 550	PBW 621	WH 1105	Mean
T1- (Ascorbic acid 100 $\mu g ml^{-1}$)	17.7	17.9	13.9	16.5	8.2	11.7	10.4	10.1	1.5	3.7	3.6	2.9
T2- (Ascorbic acid 200 $\mu g ml^{-1}$)	12.0	15.9	14.4	14.1	6.8	9.06	11.6	9.17	2.0	3.6	3.6	2.7
T3- (Arginine 1.0 mM)	9.5	18.6	16.4	14.9	5.4	12.2	9.76	9.12	0.8	4.1	2.3	2.3
T4- (Arginine 2.0 mM)	13.0	16.8	14.7	14.8	4.1	10.4	9.3	7.95	0.7	3.6	2.1	2.1
T5- (Water sprayed)	10.0	11.7	18.6	13.1	3.9	11.1	10.1	8.36	1.0	3.6	2.6	2.3
T6- (Unsprayed control)	11.2	12.0	15.4	12.9	5.8	9.8	9.06	8.24	1.2	3.5	2.0	2.2
Mean	12.2	16.3	15.6	14.4	5.7	10.7	10	8.82	1.0	3.7	2.7	2.4
CD at 5%	Varieties (A) = 0.5 Treatments (B) = 0.6 A x B = 1.1				Varieties (A) = 0.5 Treatments (B) = 0.7 A x B = 1.3				Varieties (A) = 0.1 Treatments (B) = 0.2 A x B = 0.3			

Table 9: Effect of different osmo-protectants on effective tillers per meter row length in wheat (*Triticum aestivum* L.)

Treatments	Effective tillers per meter row length									
	90 DAS		120 DAS				At Harvest			
	PBW 550	Mean	PBW 550	PBW 621	WH 1105	Mean	PBW 550	PBW 621	WH 1105	Mean
T1:- Salicylic acid (50µg ml^{-1})	72.0	72.0	103.0	104.0	103.0	103.3	106.0	109.0	105.0	106.7
T2:- Salicylic acid (75µg ml^{-1})	64.0	64.0	86.0	99.0	97.0	94.0	101.0	104.0	100.0	101.7
T3:- KNO_3 (0.5%)	73.0	73.0	105.0	109.0	107.0	107.0	110.0	119.0	108.0	112.3
T4:- KNO_3 (1%)	67.0	67.0	98.0	106.0	104.0	102.7	104.0	102.0	99.0	101.7
T5:- $ZnSO_{4.7}H2O$ (0.5%)	66.0	66.0	95.0	101.0	92.0	96.0	85.0	103.0	97.0	95.0
T6:- $ZnSO_4.7H_2O$ (1%)	61.0	61.0	95.0	86.0	97.0	92.7	84.0	99.0	98.0	93.7
T7:- Water sprayed	66.0	66.0	95.0	103.0	100.0	99.3	101.0	97.0	102.0	100.0
T8:- Unsprayed (control)	61.0	61.0	83.0	97.0	97.0	92.3	98.0	99.0	99.0	98.7
Mean	66.3	66.3	95.0	100.6	99.6	98.4	98.6	104.0	101.0	101.2
CD at 5%	Varieties (A) = 0.9 Treatments (B) = 1.4 A x B = 2.4		Varieties (A) = 0.7 Treatments (B) = 8.1 A x B = NS				Varieties (A) = 0.5 Treatments (B) = 8.5 A x B = NS			

Table 10: Effect of different antioxidants on effective tillers per meter row length in wheat (*Triticum aestivum* L.)

Treatments	Effective tillers per meter row length									
	90 DAS		120 DAS				At Harvest			
	PBW 550	Mean	PBW 550	PBW 621	WH 1105	Mean	PBW 550	PBW 621	WH 1105	Mean
T1- (Ascorbic acid 100 $\mu g ml^{-1}$)	66.7	66.7	105.0	104.0	97.3	102.0	110.0	109.0	105.0	108.0
T2- (Ascorbic acid 200 $\mu g ml^{-1}$)	73.0	73.0	98.0	98.7	103.0	99.9	104.0	103.0	100.0	103.0
T3- (Arginine 1.0 mM)	60.7	60.7	95.3	109.0	103.0	103.0	98.0	119.0	108.0	108.0
T4- (Arginine 2.0 mM)	62.7	62.7	94.7	102.0	96.7	99.1	101.0	106.0	99.0	101.0
T5- (Water sprayed)	62.4	62.4	82.7	93.7	96.7	91.6	89.0	95.0	97.0	93.1
T6- (Unsprayed control)	62.1	62.1	83.7	85.7	95.3	93.2	94.7	99.3	99.3	92.8
Mean	62.6	62.6	95.0	99.8	99.4	98.1	97.7	104.0	101.0	101.0
CD at 5%	Varieties (A) = 1.2		Varieties (A) = NS				Varieties (A) = NS			

	Treatments (B) = 1.7 A x B = 2.9	Treatments (B) = 7.0 A x B = NS	Treatments (B) = 8.2 A x B = NS

Stomatal frequency

There was a significant difference present among the varieties on stomatal frequency (both in upper and lower surface) at 60 DAS, 90 DAS and 120 DAS. PBW 621 showed maximum stomatal frequency on both the surfaces, followed by PBW 550 and minimum stomatal frequency was recorded in WH 1105 (Table 11). Difference between cultivars in terms of stomatal frequency on both adaxial and abaxial surfaces of flag leaves was also significant. Generally, all cultivars had more stomata on the adaxial compared to abaxial surface.

Among foliar spray of antioxidants, there was a significant difference present among the varieties on stomatal frequency at 60 DAS, 90 DAS and 120 DAS. PBW 621 showed maximum stomatal frequency on both the surfaces, followed by PBW 550 and minimum stomatal frequency was recorded in WH 1105. At 120 DAS, Difference between cultivars in terms of stomatal frequency on both adaxial and abaxial surfaces of flag leaves was also significant. Generally, all cultivars had more stomata on the adaxial compared to abaxial surface (Table 12).

Stomatal frequency considered as an important criterion for yield progress because it regulates the exchange of water and CO_2 between plant and the atmosphere under stress environment (Bergmann 2004) [15]. Reduction in stomatal density was found under heat stress conditions and recorded minimum stomatal density suggesting less transpiration rate under heat conditions (Rodiyati *et al* 2005) [14].

Table 11: Effect of different osmo-protectants on stomatal frequency in wheat (*Triticum aestivum* L.) at 60 DAS

(A)Varieties)	60 DAS (adaxial surface)	60 DAS (abaxial surface)	90 DAS (adaxial surface)	90 DAS (abaxial surface)	120 DAS (adaxial surface)	120 DAS (abaxial surface)
PBW 550	31.37	21.50	37.67	27.08	39.29	29.17
PBW 621	33.50	22.96	40.87	33.00	42.46	33.71
WH 1105	27.87	16.08	37.83	27.42	35.30	28.42
CD at 5%	1.74	1.18	1.20	1.18	1.29	1.32
(B) FOLIAR SPRAYS						
(Salicylic acid 50µg ml^{-1}) T1	29.11	18.22	36.78	29.78	38.33	30.33
(Salicylic acid 75µg ml^{-1}) T2	29.11	18.33	35.32	28.44	36.78	32.55
(KNO_3 0.5%) T3	25.44	17.67	35.55	28.11	36.89	31.89
(KNO_3 1%) T4	29.33	19.22	35.33	27.78	37.00	30.22
($ZnSO_4.7H_2O$ 0.5%) T5	36.67	19.33	41.11	30.55	43.22	34.44
($ZnSO_4.7H_2O$ 1%) T6	34.89	20.11	39.44	29.67	40.89	34.89
(Water sprayed) T7	30.33	20.78	36.89	28.33	38.44	32.22
(Unsprayed control) T8	32.44	18.78	39.33	30.67	41.11	31.89
CD at 5%	NS	NS	NS	NS	NS	NS

Table 12: Effect of different antioxidants on stomatal frequency in wheat (*Triticum aestivum* L.)

(A) Varieties)	60 DAS (adaxial surface)	60 DAS (abaxial surface)	90 DAS (adaxial surface)	90 DAS (abaxial surface)	120 DAS (adaxial surface)	120 DAS (abaxial surface)
PBW 550	32.00	21.72	38.55	27.17	40.27	29.61
PBW 621	33.61	22.83	40.61	33.22	42.22	34.11
WH 1105	27.89	20.33	35.55	26.67	37.28	27.83
CD at 5%	1.93	0.74	1.30	1.23	1.43	1.60
(B) Foliar Sprays						
(Ascorbic acid 100µg ml^{-1}) T1	31.67	23.55	37.11	29.22	38.77	30.00
(Ascorbic acid 200µg ml^{-1}) T2	28.88	19.67	36.67	26.44	38.22	29.78
(Arginine 1.0 Mm) T3	30.55	20.00	38.78	29.38	40.22	30.33
(Arginine 2.0 Mm) T4	31.44	20.33	38.33	29.22	40.00	30.11
(Water sprayed) T5	31.33	23.44	38.89	29.78	40.78	30.78
(Unsprayed control) T6	33.11	23.33	39.78	30.00	41.55	32.11
CD at 5%	NS	NS	NS	NS	NS	NS

Conflict of interest

All authors declare that they have no conflict of interest.

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